Attorney Docket No. C1041/7002 (AWS)

#21 / K.T. 8/7

Applicant:	Hermann Wagner and Grayson B. Lipford
Serial No:	09/241,653
Filed:	February 2, 1999
For:	METHODS FOR REGULATING HEMATOPOIESIS USING CPG-OLIGONUCLEOTIDES
Examiner:	M. Schmidt
Art Unit:	1635

Dec.

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to the Commissioner for Patents, Washington, D.C. 20231, on the 2nd day of July, 2001.

~~Monica Zombori~~
Alan Steele

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

considered 7/22/02 93

I, Grayson B. Lipford declare the following:

1. I received my B.S. from Virginia Commonwealth University/Medical College of Virginia in 1984, and my Ph.D. from Eastern Virginia Medical School in 1992. Since 1995 I have been a research group coordinator at the Institute for Medical Microbiology, Immunology and Hygiene at the Technical University of Munich, Munich, Germany. I was a research scientist at the Institute for Medical Microbiology, Immunology and Hygiene for five years prior to becoming a research group coordinator. Since 1997 I have also served as a scientific consultant to Coley Pharmaceutical Group, Inc., in the areas of vaccines and immunomodulation. For the past five years my research has been focused on properties and mechanisms of action of immunostimulatory nucleic acids, including CpG oligonucleotides. I have authored fifty peer-reviewed original research articles published in journals including *Nature*, *Journal of Immunology*, *European Journal of Immunology*, *Immunology*, *Blood*, and *EMBO Journal*, as well as five book chapters.

2. I am a co-inventor of the subject matter claimed in the above-identified application. I am familiar with the Office Action for this application, dated January 3, 2001, and the substance of the Interview held in person with the Examiner on May 30, 2001. I make this Declaration in support of the patentability of all pending claims in the application.

3. The invention relates to methods for inducing an antigen-specific immune response in a subject, as well as methods for regulating hematopoiesis, particularly erythropoiesis and thrombopoiesis, using certain nucleic acid molecules characterized by inclusion of a CpG motif. These nucleic acids are referred to as CpG oligonucleotides. The methods related to hematopoiesis are directed to methods of treating and preventing anemia and thrombocytopenia. Subjects include both human and nonhuman vertebrate subjects.

4. The Examples provided in the application describe a series of experiments performed in mice. It is my understanding that during the above-referenced Interview the Examiner expressed the desire to review a showing of a nexus between animal and human treatments. The following paragraphs are directed to establishing such a showing with respect to anemia and thrombocytopenia.

5. Anemia. Red blood cells (erythrocytes) have a limited lifespan. In health, there is a steady state balance between constant removal of senescent erythrocytes from the circulation and their replacement by newly formed erythrocytes. It is now firmly established that erythropoietin (EPO) is the primary growth factor responsible for stimulating the production of new erythrocytes in humans and nonhuman vertebrates. EPO is synthesized in the kidneys and normally is secreted in quantities sufficient to maintain a normal hematocrit. This is the basis for the widely accepted use of recombinant forms of EPO for the treatment and prevention of chronic anemia in humans susceptible to such treatment. These patients include, in particular, patients with reduced endogenous EPO production due to kidney failure, e.g., patients maintained on chronic dialysis. Anemia occurs in chronic renal failure patients because EPO production by the diseased kidneys is insufficient to maintain production of new erythrocytes. For a review, see Tong EM et al. (2001) Erythr poietin and anemia, *Semin Nephrol* 21(2):190-203 (**Exhibit 1**).

A number of studies demonstrate the efficacy of EPO treatment in animal models. While there are numerous other animal models and studies with other nonhuman vertebrates, the following are representative examples of references describing the use of EPO in various mouse models of anemia.

Zhang F et al. (1996) Anemia of chronic renal failure: characterization in the mouse and correction with human recombinant erythropoietin, *Nephron* 72(4):654-61 (**Exhibit 2**) describes the observation that inbred C57BL/6J mice made to have chronic renal failure develop a low-EPO form of anemia that was corrected by chronic administration of recombinant human EPO.

Joiner B et al. (1993) The effect of recombinant human erythropoietin treatment on tumour radiosensitivity and cancer-associated anaemia in the mouse, *Br J Cancer* 68(4):720-6 (**Exhibit 3**) describes a study showing that carcinoma NT in inbred CBA mice causes a progressive anemia that was responsive to treatment with recombinant human EPO, independent of any direct effect of EPO on the NT tumor cells.

Leroy-Viard K et al. (1991) Improvement of mouse beta-thalassemia by recombinant human erythropoietin, *Blood* 78(6):1596-602 (**Exhibit 4**) demonstrates a significant increase of hematocrit in homozygous beta thalassemic mice treated for two weeks with recombinant human EPO.

Thus, without meaning to suggest or require that CpG-based methods of treatment and prevention of anemia are limited to effects on, or effects mediated by, EPO, these studies demonstrate that various mouse models of anemia form a nexus between animal (mouse) and human treatment effects when it comes to anemia.

6. Thrombocytopenia. Platelets are circulating formed elements in the blood that are essential for stopping bleeding (hemostasis). Thus platelets, which are formed from megakaryocytes in response to thrombopoietin (TPO) and other cytokines and growth factors, are consumed and must be replenished whenever bleeding occurs. For a review, see Begley CG et al. (2000) Biologic and structural differences of thrombopoietic growth factors, *Semin. Hematol* 37(2 Suppl 4):19-27 (**Exhibit 5**). Thrombocytopenia, or depletion of platelets, is a frequently encountered problem in the setting of cancer treatment with either radiation or chem therapy, as well as in other drug-related and immunological conditions.

A number of studies dem nstrate the efficacy of TPO treatment in animal models. While there are numerous other animal models and studies with other nonhuman vertebrates, the following are representative examples of references describing the use of TPO and a related molecule, megakaryocyte growth and development factor (MGDF), in various animal models of anemia.

Wagemaker G et al. (1998) The efficacy of recombinant TPO in murine and nonhuman primate models for myelosuppresaion and stem cell transplantation, *Stem Cells* 16 Suppl 2:127-41 (**Exhibit 6**) is a review article describing the successful treatment of thrombocytopenia in myelosupressed rhesus monkeys following 5-Gy total body irradiation (TBI). A single dose of TPO administered 24 h after 5-Gy TBI effectively prevented thrombocytopenia. Similar results were reported for mice.

Ohwada A et al. (1996) In vivo adenovirus vector-mediated transfer of the human thrombopoietin cDNA maintains platelet levels during radiation- and chemotherapy-induced bone marrow suppression, *Blood* 88(3):778-84 (**Exhibit 7**) demonstrates that inbred BALB/c mice pretreated with a vector encoding TPO had higher platelet counts and more rapid platelet count recoveries following combined radiation and chemotherapy exposure (500 rads plus 1.2 mg carboplatin) than similarly exposed controls pretreated with vector alone. BALB/c mice treated with the vector encoding TPO without radiation or chemotherapy increased their platelet counts above normal, peaking at day 7.

Neumann TA et al. (2000) Megakaryocyte growth and development factor (MGDF): an Mpl ligand and cytokine that regulates thrombopoiesis, *Cytokines Cell Mol Ther* 6(1):47-56 (**Exhibit 8**) offers a review of studies performed in rodents, nonhuman primates, and humans, showing that MGDF can increase platelet counts in normal and chemotherapy- or radiotherapy-treated subjects. Notably, clinical development of PEGylated recombinant human MGDF (PEG-rHuMGDF) was halted due to the development in some patients and normal volunteers of neutralizing antibodies.

Thus, without meaning to suggest or require that CpG-based methods of treatment and prevention of thrombocytopenia are limited to effects on, or effects mediated by, TPO or MGDF, these studies demonstrate that various animal models of thrombocytopenia form a nexus between animal and human treatment effects when it comes to thrombocytopenia.

7. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 6/27/01

By: _____
Grayson B. Lipford